



Bellcomm

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date: November 4, 1971
to: Distribution
from: M. S. Feldman
subject: Review of Skylab Program Blood
Handling Procedures - Case 620

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MEMORANDUM FOR FILE

Anticipating a recommendation from the NASA Life Sciences Committee that in-flight blood sampling and return for post-flight analysis be implemented in the Skylab Program, the program office requested a review of the current pre- and post-flight blood analysis experiments to determine whether procedures used by these experiments can be utilized in flight.

There are five experiments in the Hematology and Immunology Protocol and two experiments in the Nutrition and Musculoskeletal Function Protocol that have requirements for pre- and post-flight blood analysis. Experiment Implementation Plans and Experiment Requirements Documents for each of these experiments were reviewed to determine procedures for handling and preserving blood samples prior to laboratory analysis as well as analytical procedures used in the laboratory.

Summaries of the Skylab Program medical experiment blood handling and analysis requirements are included in Attachments 1 through 7. These experiments were conceived in an environment that restricted blood withdrawal to pre-flight and post-flight phases of the mission. No in-flight blood sampling was permitted. As a result the techniques selected assume a well equipped facility at the blood withdrawal site and rapid access to a modern blood analysis laboratory. The critical timing requirements, stringent temperature control requirements, and on-site processing requirements specified by these experiments are not compatible with in-flight blood sampling.

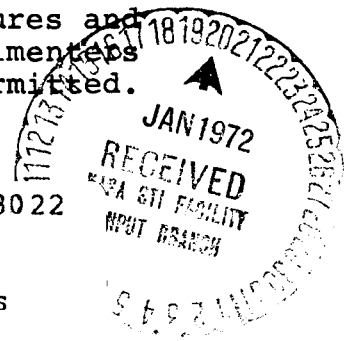
It is conceivable that different procedures and techniques would have been selected had the experimenters known that in-flight blood withdrawal would be permitted.

(NASA-CR-125367) REVIEW OF SKYLAB PROGRAM
BLOOD HANDLING PROCEDURES (Bellcomm, Inc.)

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The on-site blood handling requirements for Experiments M071-Mineral Balance and M073-Bioassay of Body Fluids are not specified in either the Experiment Requirements Document or the Experiment Implementation Plans. Since M071 analysis is used to detect stable minerals, it seems reasonable to assume that a method could be devised to preserve in-flight blood samples for post-flight detection of these minerals. Without radical changes in experiment approach, M071 appears to be the only currently approved Skylab experiment that could utilize in-flight blood sampling.


M. S. Feldman

1025-MSF-li

Attachments



Attachment 1

M111 - Crytogenetic Studies of the Blood

Purpose: Determine changes of chromosome aberration frequencies in peripheral blood leukocytes due to long duration space flight

Blood Withdrawal Schedule

1 ml on days F-30, F-20, F-14, F-1, R+0, R+7, R+14, R+21

On-Site Processing

Add heparin

Separate leukocytes

Incubate at $37^{\circ}\pm 1^{\circ}\text{C}$ in chromosome 1A Culture Medium

Laboratory Processing

Add Colchicine at proper time

Centrifuge to separate cells from medium

Suspend cells in hypotonic salt solution

Centrifuge to separate cells

Suspend cells in freshly prepared fixative

Change fixative three times

Visually analyze under microscope

Special Constraints

Timing of colchicine addition and suspension of cells in fixative is critical. These procedures must occur during metaphase stage of mitosis. Cultures must be delivered to the laboratory in less than 48 hours from the time the blood is withdrawn.

The temperature of the culture must be maintained at $37^{\circ}\pm 1^{\circ}\text{C}$ during transporation from the collection site to the laboratory.



Attachment 2

M112 - Man's Immunity - In Vitro Aspects

Purpose: Assay changes in humoral and cellular immunity.

Blood Withdrawal Schedule

10 ml on days F-30, F-7, F-3, R+0, R+7, R+21

On-Site Processing

Add heparin

Separate for two hours in $37^{\circ}\pm 1^{\circ}\text{C}$ water bath

Remove and transport plasma to laboratory at room temperature within 24 hours

Process buffy coat by nylon column technique to yield lymphocytes without PMN cells

Centrifuge to separate platelets from lymphocytes

Wash and culture lymphocytes in TC-199 tissue culture at $37^{\circ}\pm 1^{\circ}\text{C}$.

Laboratory Processing

Plasma protein analysis determined by refractometry, electrophoresis, immunoelectrophoresis, ultracentrifugation and radial immunodiffusion.

Stimulate half of lymphocyte culture with phytohemagglutinin (PHA) and perform analyses on both stimulated and unstimulated cultures.

Analysis involves use of liquid scintillation spectrometry, Coulter Counter, cinematography, and microspectrometry to determine sterility and viability of lymphocytes, morphologic response pattern, RNA and DNA synthesis rates, etc.

Special Constraints

Cultures must be delivered to laboratory in less than 23 hours from the time the blood was withdrawn.

Shock and shaking are to be avoided in handling samples

The temperature of the culture must be maintained at $37^{\circ}\pm 1^{\circ}\text{C}$ during transportation from the collection site to the laboratory.



Attachment 3

M113 - Blood Volume and Red Cell Life Span

Purpose: Determine changes in red cell mass, red cell survival and plasma volumes occurring during space flight.

Blood Withdrawal Schedule

15 ml on days F-21, R+0, R+21

2.5 ml on days F-20, F-14, F-7, F-1, R+1,
R+3, R+7, R+14

On-Site Processing

Add heparin

Add Acid Citrate Dextrose (ACD)

Inject radioactive tracers into blood

Incubate blood with isotope

Add Ascorbic Acid

Transfuse incubate into crew member

Withdraw 2.5 ml of blood from crew member 30 and
31 minutes after transfusing incubate

Add heparin

Pipette 0.7 ml blood into three 0.2 ml aliquots

Centrifuge 1.3 ml to separate plasma and red
blood cells

Freeze all samples at $-70^{\circ}\pm 10^{\circ}\text{C}$ and transport to
laboratory

Laboratory Processing

Liquid Scintillation Counter analysis to determine:

Red cell production

Red cell mass

Red cell life span

Plasma volume

Special Constraints

Samples must be maintained at $-70\pm 10^{\circ}\text{C}$ during transport to laboratory



Attachment 4

M114 - Red Blood Cell Metabolism

Purpose: Determine effect of space flight on red cell metabolism and membrane integrity and determine causes of any changes.

Blood Withdrawal Schedule

10 ml on days F-30, F-7, F-1, R+0, R+1, R+14

On-Site Processing

Add heparin to 8 ml of 10 ml sample

Add Acid Citrate Dextrose (ACD) to 2 ml of sample

Freeze ACD sample at $-70 \pm 10^{\circ}\text{C}$

Freeze 5 ml of 8 ml heparinized sample at $-70 \pm 10^{\circ}\text{C}$.

Chill 3 ml of heparinized sample to $4^{\circ} \pm 2^{\circ}\text{C}$

Laboratory Processing

All frozen samples analyzed by spectrophotometer

Chilled sample analyzed by incubation lysis, titration, and spectrophotometer

Special Constraints

All samples must arrive at laboratory within 48 hours after blood withdrawal

Frozen samples must be maintained at $-70 \pm 10^{\circ}\text{C}$

Chilled samples must be maintained at $4^{\circ} \pm 2^{\circ}\text{C}$



Attachment 5

M115 - Special Hematologic Effects

Purpose: Evaluate effects of space flight on critical physiochemical hematologic parameters relative to the maintenance of homeostasis and provide essential hematologic data to lend continuity to the hematology/immunology experiment.

Blood Withdrawal Schedule

7 ml on days F-30, F-14, F-7, F-1, R+0, R+3, R+7, R+21

3.5 ml on days R+1, R+14

On-Site Processing

Add heparin to 3.5 ml of 7 ml sample

Add ethylenediaminetetracetic acid (EDTA) to 3.5 ml of 7 ml sample

Use Coulter Counter to determine red cell count, white cell count, and platelet count using part of EDTA treated sample.

Use centrifuge to determine hematocrit using part of EDTA treated sample.

Add reagent to part of EDTA treated sample and use spectrophotometer to determine hemoglobin content of red blood cell mass

Obtain stained slide of EDTA treated sample, determine reticulocyte count, differential, and morphology using microscope.

Use part of heparinized blood to determine red blood cell critical volume and volume distribution using Coulter Counter

Use part of heparinized blood and Fragillograph to determine fragility of cell membrane by osmotic rupture in acid solution and in saline solution.

Chill part of heparinized blood to $4^{\circ}\pm 2^{\circ}\text{C}$ for transport to laboratory

Centrifuge part of heparinized blood to obtain red blood cells, hemolyze and wash, and transport to laboratory at ambient temperature

Prepare part of heparinized blood for microanalysis and transport to laboratory at ambient temperature.



Laboratory Processing

Heparinized blood transported at $4^{\circ}\pm 2^{\circ}\text{C}$ will be analyzed by chromatography, electrophoresis and differential centrifugation techniques.

Hemolyzed red blood cells will be analyzed by flame photometry

Samples prepared for microanalysis will be analyzed by microspectrophotometer, electron microprobe, and electron microscope.

Special Constraints

None specified.



Attachment 6

M071 - Mineral Balance

Purpose: Determine effects of space flight on the muscle and skeletal body systems by quantitative assessment of gains or losses of pertinent biochemical constituents.

Blood Withdrawal Schedule

15 ml on days F-14, F-7, F-1, R+0, R+7, R+14

On-Site Processing

Not specified other than chill to 4°C

Laboratory Processing

Flame photometry for calcium, phosphorus, magnesium, alkaline phosphatase, sodium, and potassium

Titration for total protein

Electrophoresis for electrophoresis pattern

Spectrophotometry for sugar

Column chromatography for hydroxyproline

Special Constraints

None specified



Attachment 7

M073 - Bioassay of Body Fluids

Purpose: Evaluate the endocrinological adaptation resulting from exposure to space flight environment

Blood Withdrawal Schedule

25 ml on days F-21, F-20, F-14, F-1, R+0, R+1, R+3, R+21

On-Site Processing

Not specified

Laboratory Processing

Radio Immuno Assay for angiotension II, renin, parathyroid hormone, thyrocalcitonin, calcitonion, thyroxine, and ACTH

Immunodiffusion for ADH

Freeze point depression for osmolality

Cortisol binding globulin for 17-hydroxycorticosterone

Barium sulfate for extracellular fluid volume

Deuterium oxide for total body water

Special Constraints

None specified.



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